

IN THE CLAIMS

1. (Previously Presented) A method for ligation of a plurality of DNA segments to obtain a ligation product that comprises sequences from each of said DNA segments in a predetermined order, said method comprising:

a) providing at least three DNA molecules, each comprising a DNA segment and a vector segment, wherein each DNA segment is adjacent to one or two other DNA segments in the ligation product, wherein each DNA segment comprises a first region having sequence identity with a first adjacent DNA segment and a second region having sequence identity with a second adjacent DNA segment, if present, and wherein each vector segment comprises a selectable marker and/or a counter-selectable marker;

b) cleaving each DNA vector to produce a DNA segment with one or two ligatable ends, each said ligatable end comprising at least a portion of the region having sequence identity with an adjacent DNA segment; wherein at least one segment comprises two such ligatable ends after cleavage and wherein at least two DNA segments comprise exactly one such ligatable end,

c) simultaneously ligating each DNA segment to the adjacent DNA segment or segments, wherein the DNA segment with one or two ligatable ends is not purified prior to said ligating; and

d) selecting a ligation product comprising sequences from each of said DNA segments in a predetermined order, wherein said selection is based on the presence in a vector comprising the ligation product of a selectable marker and counter selectable marker of at least one of said three DNA vectors.

2. (Currently Amended) A method for ligation of a plurality of DNA segments to obtain a ligation product that comprises sequences from each of said DNA segments in a predetermined order, said method comprising:

a) providing a Type 1 DNA molecule, a Type 2 DNA molecule and at least one Type 3 DNA molecule, each comprising a DNA segment that is adjacent to one or two other segments in the final ligation product, wherein

i) said Type 1 DNA molecule comprises a first DNA segment, a first selectable marker, a first counter-selectable marker, a first cleavage site, and a second cleavage site,

wherein cleavage of said second cleavage site produces a single-strand overhang in said first DNA segment which is ligatable to a single-strand overhang of an adjacent segment;

ii) said Type 2 DNA molecule comprises a second DNA segment, a second selectable marker, a second counter-selectable marker, a third cleavage site, and a fourth cleavage site, wherein cleavage of said fourth cleavage site produces a single-strand overhang in said second DNA segment which is ligatable to a single-strand overhang of an adjacent segment;

iii) each said Type 3 DNA molecule comprises a DNA segment, a third counter-selectable marker, a 5-prime cleavage site and a 3-prime cleavage site, wherein said 5-prime cleavage site, upon cleavage, produce a single-strand overhang in the segment that is ligatable to a single-strand overhangs of an adjacent segment, and said 3-prime cleavage site, upon cleavage, produce a single-strand overhang in the segment that is ligatable to a single-strand overhangs of a different adjacent segment;

wherein said first and second selectable markers are different;

wherein said first, second and third counter-selectable markers are independently selected and are the same or different;

wherein said first and third cleavage sites are the same or are compatible;

wherein said second and fourth cleavage sites are independently selected and are the same or are different; and,

wherein each 5-prime and 3-prime cleavage site is independently selected in each Type 3 DNA molecule and are the same or are different;

b) cleaving each DNA molecule at the first, second, third, and fourth cleavage sites, at the 5-prime cleavage site(s) and at the 3-prime cleavage site(s); and

c) without purifying the products of said cleaving of each DNA molecule, ligating the resulting fragments to each other thereby producing a ligation product that comprises sequences from each of said DNA segments in a predetermined order.

3. (Original) The method of claim 2 further comprising the steps

d) transforming cells with ligation products produced in step (c); and

e) selecting transformants that express said first and second selectable markers and do not express said first, second, or third counter-selectable marker.

4. (Original) The method of claim 3 further comprising the step:
 - f) isolating the ligation product comprising sequences from each of said DNA segments in a predetermined order from the transformants or their progeny.
5. (Original) The method of claim 2 wherein said first and second selectable markers are genes conferring drug resistance.
6. (Currently Amended) The method of claim 2 wherein said first, second and third counter-selectable markers are selected from the group consisting of ccdB (anti-DNA gyrase protein), sacB (sucrose sensitivity), araB (ribulose sensitivity), and tetAR (tetracycline resistance/fusaric acid hypersensitivity).
7. (Original) The method of claim 2 wherein
 - a) said first and third cleavage sites are the same;
 - b) said second and fourth cleavage sites are the same;
 - c) the 5-prime cleavage site of at least one Type 3 DNA molecule is the same as the 3-prime cleavage site of the same Type 3 DNA molecule; and/or
 - d) the 5-prime cleavage site of at least one Type 3 DNA molecule is the same as the 5-prime cleavage site of a different Type 3 DNA molecule.
8. (Original) The method of claim 2 wherein
 - a) said first and third cleavage sites are sites cleaved by a Type IIS restriction enzyme;
 - b) said second and fourth cleavage sites are sites cleaved by a Type IIS restriction enzyme; and/or
 - c) said 5-prime and 3-prime cleavage sites of at least one Type 3 DNA molecule are sites cleaved by a Type IIS restriction enzyme.
9. (Original) The method of claim 2 wherein the first, second, third, fourth, 5-prime and 3-prime cleavage sites are sites cleaved by a Type IIS restriction enzyme.

10. (Original) The method of claim 2 wherein the first, second, third, fourth, 5-prime and 3-prime cleavage sites are sites cleaved by the same Type IIS restriction enzyme.
11. (Original) The method of claim 2 wherein the DNA segments of the Type 1, Type 2 and Type 3 DNA molecules comprise sequences encoding a polypeptide segment of a polyketide synthase.
12. (Original) The method of claim 2 wherein the DNA segments of the Type 1, Type 2 and Type 3 DNA molecules comprise sequences encoding a polyketide synthase domain.
13. (Original) The method of claim 2 wherein the DNA molecules cleaved in step (b) are cleaved in the same container.
14. (Currently Amended) A composition comprising:
 - a) an unpurified cleavage product of:
 - i) a Type 1 DNA molecule, said DNA molecule comprising a first DNA segment, a first selectable marker, a first counter-selectable marker, a first cleavage site, and a second cleavage site; wherein cleavage of said second cleavage site produces a single-strand overhang in said first segment which is ligatable to a single-strand overhang of an adjacent segment;
 - ii) a Type 2 DNA molecule, said DNA molecule comprising a second DNA segment, a second selectable marker, a second counter-selectable marker, a third cleavage site, and a fourth cleavage site wherein cleavage of said fourth cleavage site produces a single-strand overhang in said first segment which is ligatable to a single-strand overhang of an adjacent segment;
 - iii) at least one Type 3 DNA molecule, said DNA molecule comprising a DNA segment, a third counter-selectable marker, a 5-prime cleavage site and a 3-prime cleavage site,
wherein said 5-prime and 3-prime cleavage sites, upon cleavage, produce single-strand overhangs in the segment that are ligatable to a single-strand overhangs of each of two adjacent segments;

wherein said first and second selectable markers are different;

wherein said first, second and third counter-selectable markers are independently selected and are the same or different; wherein said first and third cleavage sites are the same or are compatible;

wherein said second and fourth cleavage sites are independently selected and are the same or are different; and,

wherein each 5-prime and 3-prime cleavage site is independently selected; and
b) a DNA ligase.

15. (Original) The composition of claim 14 comprising at least two Type 3 DNA molecules.
16. (Original) The composition of claim 14 comprising an endonuclease that cleaves at the first, second, third, or fourth cleavage sites or at one or more 5-prime or 3-prime cleavage sites.
17. (Original) The composition of claim 16 wherein the endonuclease cleaves at the first, second, third, and fourth cleavage sites and at one or more 5-prime or 3-prime cleavage sites.
18. (Original) The composition of claim 16 that contains at least two Type 3 DNA molecules comprising 5-prime or 3-prime cleavage sites and wherein the endonuclease cleaves at the third and fourth cleavage sites and at the 5-prime and 3-prime cleavage sites of said Type 3 DNA molecules.
19. (Currently Amended) [[A]] The composition of claim 14, wherein, after a sufficient amount of time for a ligation reaction using said DNA ligase, a product is formed, the product comprising:
 - the first DNA segment immediately upstream of the second DNA segment,
 - wherein the second DNA segment is immediately upstream of the DNA segment from at least one Type 3 DNA molecule; and
 - at least one selectable marker.

20-30. (Cancelled)

31. (Previously Presented) The method of claim 1 wherein the selectable markers in (a) are sequences encoding a protein that confers drug resistance to a host, and the selection in step (d) is based on the presence in a vector comprising the ligation product of two different selectable markers, wherein the two different selectable markers are associated with two different vectors in step (a).

32. (Currently Amended) The method of claim 1 wherein the selectable marker provides resistance to a drug selected from the group consisting of carbenicillin, tetracycline, kanamycin, streptomycin, ~~[[or]]~~and chloramphenicol.

33. (Previously Presented) The method of claim 31 wherein the selection in step (d) is based on the absence in a vector comprising the ligation product of a counter-selectable marker present in one or more of the vectors in (a).

34. (Previously Presented) The method of claim 31 wherein the cleaving of step (b) comprises digestion with a Type IIS restriction enzyme.

35. (Previously Presented) A method for ligation of a plurality of DNA segments to obtain a ligation product that comprises sequences from each of said DNA segments in a predetermined order, said method comprising:

a. providing at least three different DNA vectors, each comprising a DNA segment,

wherein each said segment is adjacent to at least one other segment in the ligation product,

wherein each segment comprises a region(s) having sequence identity with the adjacent DNA segment(s), and

wherein each of said DNA vectors comprises a selectable marker such that;

- i. the first DNA vector comprises a first selectable marker
- ii. the second DNA vector comprises a second selectable marker
- iii. the third DNA vector comprises a third selectable marker

b. cleaving each DNA vector to produce a linear DNA molecule, each linear DNA molecule comprising a DNA segment with at least one end ligatable to an adjacent DNA segment,

wherein the first DNA vector is cleaved so that the resulting linear DNA molecule comprises a DNA segment that is covalently associated with first vector sequences comprising sequence encoding the first selectable marker, and the second DNA vector is cleaved so that the resulting linear DNA molecule comprises a DNA segment that is covalently associated with second vector sequences comprising sequence encoding the second selectable marker;

c. simultaneously ligating each segment to the adjacent segment or segments and ligating the first vector sequence to the second vector sequence, wherein said DNA segments from said at least three different DNA vectors are not purified prior to said ligating[[:]], thereby producing a closed circular DNA molecule comprising said segments, said first selectable marker, and said second selectable marker; and

d. selecting a ligation product comprising sequences from each of said DNA segments in a predetermined order, wherein said selection is based on the presence in the ligation product of the first selectable marker, the second selectable marker, or both the first and second selectable markers[[:]].

36. (Currently Amended) The method of claim 35 wherein said selecting is based on the presence in the ligation product of both the first and second selectable markers[[:]].

37. (Currently Amended) The method of claim 36 wherein the selectable markers provide resistance to a drug selected from the group consisting of carbenicillin, tetracycline, kanamycin, streptomycin, [[or]]and chloramphenicol.

38. (Previously Presented) The method of claim 35 wherein the cleaving of step (b) comprises digestion with a Type IIS restriction enzyme.

39. (Currently Amended) The method of claim 35 wherein at least one of the selectable markers is a counter selectable marker selected from the group consisting of *ccdB* (anti-DNA gyrase protein), *sacB* (sucrose sensitivity), *araB* (ribulose sensitivity), and *tetAR* (tetracycline resistance/fusaric acid hypersensitivity).